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- (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).
- (72) Inventors: XIANG, YiBin; 821 Main Street, Acton, MA 01720 (US). BEMIS, Jean; 256 Appleton Street, Arlington, MA 02174 (US). MCKEW, John; 58 Varnum Street, Arlington, MA 02174 (US). KAILA, Neelu; 2 Course Brook Lane, Natick, MA 01760 (US).
- (74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).

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(54) Title: INHIBITORS OF PHOSPHOLIPASE ENZYMES

(57) Abstract

Compounds having a chemical formula selected from the group consisting of formulae (I), (II) and (III) or a pharmaceutically acceptable salt wherein: A is independent of any other group and is selected from the group consisting of -CH2- and -CH₂-CH₂-; B is independent of any other group and is selected from the group consisting -(CH₂)_n-,

CCH2/ n^- , -(CH2O) n^- , -(CH2) n^- , -(CH2) n^- , -(CH=CH) n^- , -(C=C) n^- , -CON(R₆)-, -N(R₆)CO-, -O-, -S- and -N(R₆)-; R₂ is independent of any other R group and is selected from the group consisting of -H, -COOH, -COR₅, -CONR₅R₆, -(CH₂) n^- W-(CH₂) n^- W-CH₂) n^- W-R₅, -COH₂, -COH₃, -CONR₅R₆, -(CH₂) n^- W-(CH₂) n^- W-R₅, -COOH, -COR₅, -CONR₅R₆, -(CH₂) n^- W-(CH₂) n^- W-R₅, -COOH, -COR₅, -CONR₅R₆, -(CH₂) n^- W-(CH₂) n^- W-R₅, -COOH, -COR₅, -CONR₅R₆, -(CH₂) n^- W-(CH₂) n^- W-R₅, -CH₂) n^- W-R₅, -Z-R₅, C₁-C₁₀ alkyl, alkenyl and substituted aryl; which inhibit the activity of phospholipase enzymes, particularly cytosolic phospholipase A₂. Pharmaceutical compositions comprising such compounds and methods of treatment using such compositions are also disclosed.

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INHIBITORS OF PHOSPHOLIPASE ENZYMES

Background of the Invention

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The present invention relates to chemical inhibitors of the activity of various phospholipase enzymes, particularly phospholipase A_2 enzymes.

Leukotrienes and prostaglandins are important mediators of inflammation. Leukotrienes recruit inflammatory cells such as neutrophils to an inflamed site, promote the extravasation of these cells and stimulate release of superoxide and proteases which damage the tissue. Leukotrienes also play a pathophysiological role in the hypersensitivity experienced by asthmatics [See, e.g. B. Samuelson et al., Science, 237:1171-76 (1987)]. Prostaglandins enhance inflammation by increasing blood flow and therefore infiltration of leukocytes to inflamed sites. Prostaglandins also potentiate the pain response induced by stimuli. Prostaglandins and leukotrienes are unstable and are not stored in cells, but are instead synthesized [W. L. Smith, Biochem. J., 259:315-324 (1989)] from arachidonic acid in response to stimuli. Likewise arachidonic acid is not free in cells but is released from the sn-2 position of membrane phospholipids by phospholipase A2 (hereinafter PLA2). The reaction catalyzed by PLA2 is believed to represent the rate-limiting step in the process of lipid mediated biosynthesis. When the phospholipid substrate of PLA2 is of the phosphotidyl choline class with an ether linkage in the sn-1 position, the lysophospholipid produced is the immediate precursor of platelet activating factor (hereafter called PAF), another potent mediator of inflammation [S.I. Wasserman, Hospital Practice, 15:49-58 (1988)]. Consequently the direct inhibition of the activity of PLA2 has been suggested as a useful mechanism for a therapeutic agent, i.e., to interfere with the inflammatory response. [See, e.g., J. Chang et al, <u>Biochem. Pharmacol.</u>, <u>36</u>:2429-2436 (1987)].

A family of PLA₂ enzymes characterized by the presence of a secretion signal sequenced and ultimately secreted from the cell have been sequenced and structurally defined. These secreted PLA₂s have an approximately 14 kD molecular weight and contain seven disulfide bonds which are necessary for activity. These PLA₂s are found in large quantities in mammalian pancreas, bee venom, and various snake venom. [See, e.g., references 13-15 in Chang et al, cited above; and E. A. Dennis, <u>Drug Devel. Res.</u>, 10:205-220 (1987).] However, the pancreatic enzyme is believed to serve a digestive function and, as such, should not be important in the production of the inflammatory mediators whose production must be tightly regulated.

The primary structure of the first human non-pancreatic PLA_2 has been determined. This non-pancreatic PLA_2 is found in platelets, synovial fluid, and spleen and is also a

secreted enzyme. This enzyme is a member of the aforementioned family. [See, J. J. Seilhamer et al, J. Biol. Chem., 264:5335-5338 (1989); R. M. Kramer et al, J. Biol. Chem., 264:5768-5775 (1989); and A. Kando et al, Biochem. Biophys. Res. Comm., 163:42-48 (1989)]. However, it is doubtful that this enzyme is important in the synthesis of prostaglandins, leukotrienes and PAF, since the non-pancreatic PLA₂ is an extracellular protein which would be difficult to regulate, and the next enzymes in the biosynthetic pathways for these compounds are intracellular proteins. Moreover, there is evidence that PLA₂ is regulated by protein kinase C and G proteins [R. Burch and J. Axelrod, Proc. Natl. Acad. Sci. U.S.A., 84:6374-6378 (1989)] which are cytosolic proteins which must act on intracellular proteins. It would be impossible for the non-pancreatic PLA₂ to function in the cytosol, since the high reduction potential would reduce the disulfide bonds and inactivate the enzyme.

A murine PLA₂ has been identified in the murine macrophage cell line, designated RAW 264.7. A specific activity of 2 µmols/min/mg, resistant to reducing conditions, was reported to be associated with the approximately 60 kD molecule. However, this protein was not purified to homogeneity. [See, C. C. Leslie et al, <u>Biochem. Biophys. Acta.</u>, 963:476-492 (1988)]. The references cited above are incorporated by reference herein for information pertaining to the function of the phospholipase enzymes, particularly PLA₂.

A cytosolic phospholipase A₂ (hereinafter "cPLA₂") has also been identified and cloned. See, U.S. Patent Nos. 5,322,776 and 5,354,677, which are incorporated herein by reference as if fully set forth. The enzyme of these patents is an intracellular PLA₂ enzyme, purified from its natural source or otherwise produced in purified form, which functions intracellularly to produce arachidonic acid in response to inflammatory stimuli.

Now that several phospholipase enzymes have been identified, it would be desirable to identify chemical inhibitors of the action of enzymes, which inhibitors could be used to treat inflammatory conditions. However, there remains a need in the art for an identification of effective anti-inflammatory agents for therapeutic use in a variety of disease states.

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Summary of the Invention

The present invention provides compounds having a chemical formula selected from the group consisting of:

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$$R_1$$
 R_2
 R_3

$$R_1$$
 R_2
 R_3
 R_5

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and

$$\bigcap_{R_1} \bigcap_{R_3} \bigcap_{R_5} \bigcap_{R_5}$$

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or a pharmaceutically acceptable salt thereof, wherein:

A is independent of any other group and is selected from the group consisting of -CH₂- and -CH₂-CH₂-;

B is independent of any other group and is selected from the group consisting of $-(CH_2)_n$ -, $-(CH_2O)_n$ -, $-(CH_2S)_n$ -, $-(CCH_2)_n$ -, $-(CCH_2)_n$ -, $-(CCH_2CH)_n$ -, $-(CEC)_n$ -,

 R_1 is independent of any other R group and is selected from the group consisting of $-X-R_6$, -H. -OH, halogen, -CN, -NO₂, C₁-C₅ alkyl, alkenyl, alkinyl, aryl and substituted aryl;

 R_2 is independent of any other R group and is selected from the group consisting of -H, -COOH, -COR₅, -CONR₅R₆, -(CH₂)_n-W-(CH₂)_m-Z-R₅, -(CH₂)_n-W-R₅, -Z-R₅, C₁-C₁₀ alkyl, alkenyl and substituted aryl;

 R_3 is independent of any other R group and is selected from the group consisting of -H, -COOH, -COR₅, -CONR₅R₆, -(CH₂)_n-W-(CH₂)_m-Z-R₅, -(CH₂)_n-W-R₅, -Z-R₅, C₁-C₁₀ alkyl, alkenyl and substituted aryl;

 R_4 is independent of any other R group and is selected from the group consisting of -H, -OH, -OR₆, -SR₆, -CN, -COR₆, -NHR₆, -COOH, -CONR₆R₇, -NO₂, -CONHSO₂R₈, C₁-C₅ alkyl, alkenyl and substituted aryl;

 R_5 is independent of any other R group and is selected from the group consisting of -H, -OH, -O(CH₂)_nR₆, -SR₆, -CN, -COR₆, -NHR₆, -COOH, -NO₂, -COOH, -CONR₆R₇, -CONHSO₂R₈, C₁-C₅ alkyl, alkenyl, alkinyl, aryl, substituted aryl, -CF₃, -CF₂CF₃ and

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 R_6 is independent of any other R group and is selected from the group consisting of -H, C_1 - C_5 alkyl, alkenyl, alkinyl, aryl and substituted aryl;

 R_7 is independent of any other R group and is selected from the group consisting of -H, C_1 - C_5 alkyl, alkenyl, alkinyl, aryl and substituted aryl;

 R_8 is independent of any other R group and is selected from the group consisting of C_1 - C_3 alkyl, aryl and substituted aryl;

 R_9 is independent of any other R group and is selected from the group consisting of -H, -OH, a halogen, -CN, -OR₆, -COOH, -CONR₆R₇, tetrazole, -CONHSO₂R₈, -COR₆, -(CH₂)_nCH(OH)R₆ and -(CH₂)_nCHR₆R₅;

 R_{10} is independent of any other R group and is selected from the group consisting of -H, -OH, a halogen, -CN, -OR₆, -COOH, -CONR₆R₇, tetrazole, -CONHSO₂R₈, -COR₆, -(CH₂)_nCH(OH)R₆ and -(CH₂)_nCHR₆R₅;

W is, independently each time used including within the same compound, selected from the group consisting of -O-, -S-, -CH₂-, -CH=CH-, -C \equiv C- and -N(R₆)-;

X is independent of any other group and is, independently each time used including within the same compound, selected from the group consisting of -O-, -S- and -N(R6)-;

Z is independent of any other group and is, independently each time used including within the same compound, selected from the group consisting of -CH₂-, -O-, -S-, -N(R_6)-, -CO-, -CON(R_6)- and -N(R_6)CO-;

m is, independently each time used including within the same compound, an integer from 0 to 4; and

n is independent of m and is, independently each time used including within the same compound, an integer from 0 to 4.

Preferably, the compounds of the invention have phospholipase enzyme inhibiting activity. Other preferrred embodiments include compounds having the following chemical formula:

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$$R_1$$
 R_2
 R_3

compounds having the following chemical formula:

$$R_1$$
 R_3 R_5 ; and

compounds having the following chemical formula:

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$$R_1$$
 R_3 R_4

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In particularly preferred embodiments, A is -CH₂- and R₂ is $-(CH_2)_n-W-(CH_2)_m-ZR_5.$ These preferred compounds includes those wherein n is 1, m is 1, W is -S- and Z is -CO-; those wherein R₅ is -NHR₆; those wherein R₆ is a substituted aryl group and those wherein said aryl group is substituted with one or more substituents independently selected from the group consisting of a halogen, -CF₃,

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-CF₂CF₃, -(CH₂)_pCOOH, -(CH₂)_pCH₃, -O(CH₂)_pCH₃, -(CH₂)_pOH, -(CH₂)_pS(C₆H₆), -(CH₂)_pCONH₂ and -CHR₁₁COOH, wherein R₁₁ is selected froup the group consisting of alkyl, alkenyl, alkynyl, -(CH₂)_pOH, and -O(CH₂)_pCH₃, and wherein p is an integer from 0 to 4. Other preferred comounds include those wherein R₁ is selected from the group

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consisting of -H and -OCH₂(C_6H_6) and R_3 is -COR₅, R_5 is -OCH₂R₆ and R_6 is a substituted aryl group. In particularly preferred compounds, said aryl group is substituted with one or more substituents selected from the group consisting of -CF₃, -CF₂CF₃ and -C(CH₃)₂CH₂CH₃.

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The present invention also provides for a method of inhibiting the phospholipase enzyme activity of an enzyme, comprising administering to a mammalian subject a therapeutically effective amount of a compound of the present invention. Methods of treating an inflammatory condition, comprising administering to a mammalian subject a therapeutically effective amount of a compound of the present invention are also provided. Pharmaceutical compositions comprising compounds of the present invention and a pharmaceutically acceptable carrier are also provided.

Pharmaceutically acceptable salts of the compounds of the compounds described herein are also part of the present invention and may be used in practicing the compounds and methods disclosed herein.

5 Brief Description of the Figures

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Figs. 1-13 depict schemes for synthesis of compounds of the present invention. The depicted schemes are described in further detail below.

Detailed Description of Preferred Embodiments

As used herein: "halogen" includes chlorine, fluorine, iodine and bromine; "alkyl", "alkenyl" and "alkinyl" include both straight chain and branched moieties; "aryl" includes single and multiple ring moieties; and "substituted" denotes the presence of one or more similar of dissimilar substituent groups of any character.

Preferred compounds of the present invention are disclosed in Tables I-VI below.

Methods for synthesis of the compounds listed in Tables I-VI are described below.

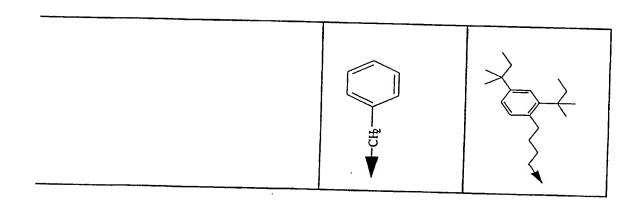
Compound Nos. in the tables correspond to example numbers below describing synthesis of that particular compound.

Tables I-VI also report data for the listed compounds in the "LysoPC" assay and the Coumarine assay (see Example 88 below). In the data columns of the tables, assay results are reported as an " IC_{50} " value, which is the concentration of a compound which inhibits 50% of the activity of the phospholipase enzyme in such assay. Where no numerical IC_{50} value appears, "NA" denotes that inhibitory activity was not detected from such compound in the corresponding assay and a blank box denotes that the compound was not tested in such assay as of the time of filing of the present application.

	IC _{s0} (µM) Lyso Cou- PC marine	6
	. R.	H000——————————————————————————————————
R ₂	R,	· 보-
	R,	
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	No.	7

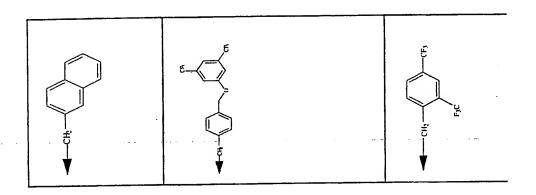
7
SUBSTITUTE SHEET (RULE 26)

4		52	٠
6.5	VV.	4.3	2.0
C00H	←CH ₂ O CF ₃	NH NH	O CH ₃ O NH



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ε n	4	10	
L		, ,	9

13	3.8	6.5	28
2.1	0.11	0.081	0.33
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SUBSTITUTE SHEET (RULE 26)

33	01	12	4
	4	0.5	6:1
F. Z	CH,O S NH		
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	-СН,	-СН,СН,	£
	-Сн³0-		
= .	13	13	4

SUBSTITUTE SHEET (RULE 26)

13	50
6.5	6.5
	COOH NH COOH
CH ₂	
15	

11
SUBSTITUTE SHEET (RULE 26)

THE PARTY OF THE P

	μМ)	Con-	marine	9
	IС ₅₀ (µМ)	Lyso	PC	0.32
	R_2			NH
R	R,			
	No.			17

01	4	O	4.5
0.28	0.21	0.28	0.29
H _S C NH COOH	HOOD HN NH	H ₂ CO NH	HO COOH

- 1				
	18	19	20	21

ري د	ν.	2.5	01
0.10	0.95	1.6	. 1.3
HOCOOH	H0003	HOOO HIN COOH	H ₂ COOH

7	ϵ	4	δ
2	7	7	7
	<u> </u>		

8	13	!
1.2	2.3	1
H,CO COOH	H), COOH	H,CO NH OH

|--|

1		
	28	44
H ₃ CO NH COOCH ₃ OCH ₃	NH OCH 3	H,CO NH CF,

29	30	31

ν,	v	>50
3.8	2.6	24
H ₃ CO NH COOH	H ₃ CO NH OOH	H ₃ CO NH

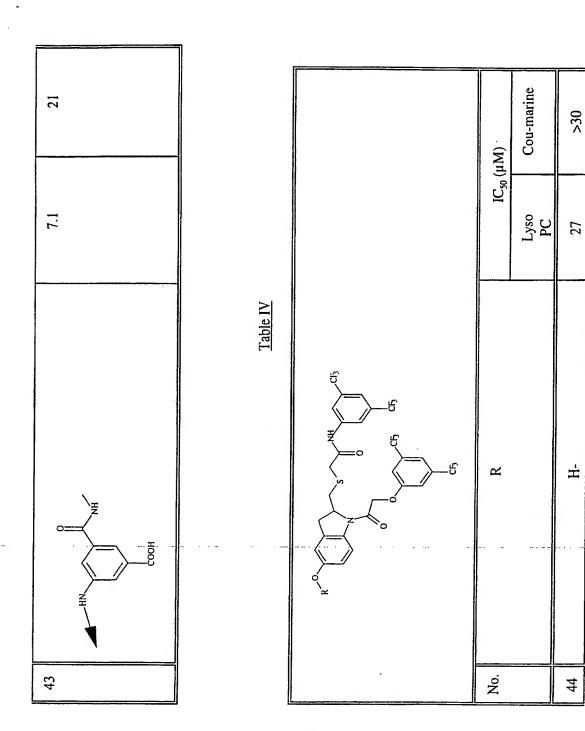
32	33	34
		·

58	4
9.1	2.3
CC NH-S OH	H ₂ CO NH
	H-
35	36

·	IC ₅₀ (µМ)	Lyso Cou-marine PC	7.6 >30	6.9 >50
O C C S	R		НО-	HOOO HOOO
	No.		37	38

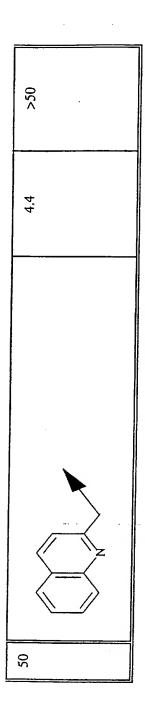
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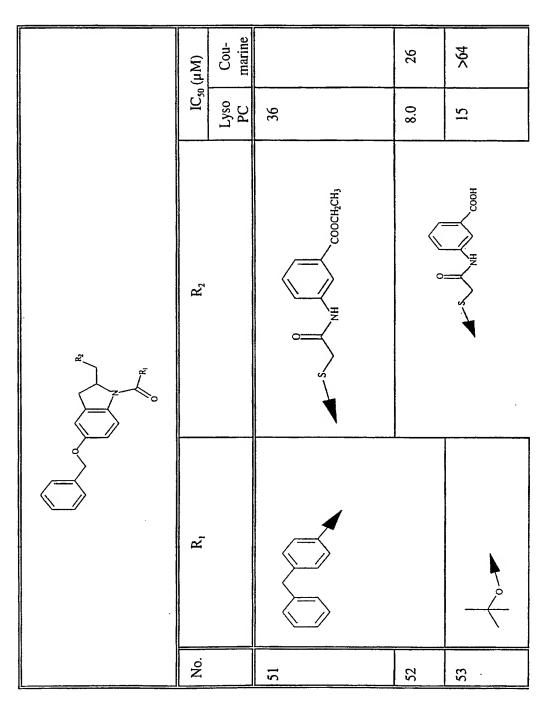
	=	22	41
4.3	6.2	2.2	7.8
HOOD	HIN CH ₃	HOOO	HOOO
39	40	14	42



21

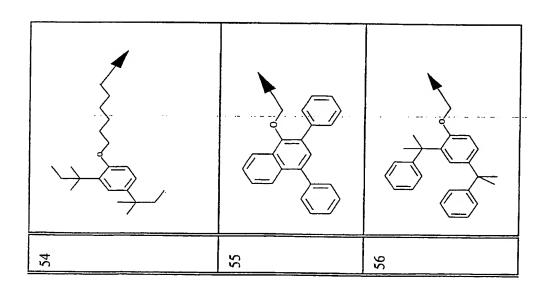
۲C	01	16	5.5	>50
0.37	0.71	1.6	0.3	40
Br				CH ₃ -
45	46	47	48	49





24

41	12	5
0.23	0.45	0.47



25

∞	4	>30	>30
0.26	0.56	8.7	4.6
		H ₃ CO HN	
OCH,			
57	58	59	09

>20	∞	
12.1	1.7	
		,
61	62	63

27

	>64		9	10	>50	9
	17.6		2.3	0.22	>50	19.4
	HNOOD	HOOD		HOOD	CONHCH ₃	H _J CO HIN
	CH ³ (CH ³) ³ O-					
7	5	65	99		89	69

29

		IC ₅₀ (μΜ)	Cou- marine	>64	9	
		IC	Lyso PC		 1.9	
Table VI		R,		HOOD		
	R ₁	R ₂				
		R				
		No.		72	73	

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5	6.2	4.5	2.5	3
1.1	7.0			
	H,CO NH	нооо		
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.>50	>50	>50
>50	23	. 21
NII	NH-S-CH	SHIN SHIN
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Н-		
83	84	85